Tansley review

Bioenergy from plants and the sustainable yield challenge

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Summary

Bioenergy from plants, particularly from perennial grasses and trees, could make a substantial contribution to alleviation of global problems in climate change and energy security if high yields can be sustained. Here, yield traits in a range of key bioenergy crops are reviewed, from which several targets for future improvement can be identified. Some are already the focus of genetically modified (GM) and non-GM approaches. However, the efficient growth strategies of perennial bioenergy crops rely on newly assimilated and recycled carbon and remobilized nitrogen in a continually shifting balance between sources and sinks. This balance is affected by biotic (e.g. pest, disease) and abiotic (e.g. drought) stresses. Future research should focus on three main challenges: changing (photo)thermal time sensitivity to lengthen the growing season without risking frost damage or limiting remobilization of nutritional elements following senescence; increasing aboveground biomass without depleting belowground reserves required for next year's growth and thus without increasing the requirement for nutrient applications; and increasing aboveground biomass without increasing water use.

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I. Introduction

Two main drivers have pushed renewable energy production to the top of global agendas: climate change and energy security. Energy consumption worldwide increased 13-fold in the 20th century, tripling since 1960, which is faster than the increase in population size (Hein, 2005). Concerns heighten about how such rising demands can continue to be met by finite

Table 1 Definitions of bioenergy terms as used in this review

Term	Definition	Comment
Bioenergy	Production of any form of renewable energy from biological sources	Focus on plants, but algal, animal and microbial sources are also important
Biomass	Biological mass from which energy can be produced	Includes not only harvestable mass but also residues
Lignocellulose	Subset of biomass that comprises the structural components (e.g. cell walls)	
Bioenergy crops	A generic term embracing crops grown for both power and transport markets	The terms 'bioenergy' and 'biofuels' are often confused, but a distinction is required (see below)
Biomass crops	Crops grown for biomass production for either market	
Biofuel crops	Crops grown for transport fuels	Often also referred to as first or second generation (see below)
Biopower crops	Crops grown for heat or power	No term is in common use for crops grown for power generation, but this can be adopted
First generation	Crop/fuel chains based on existing conversion technologies	Currently exclusively from sugar, starch or oil crops, also grown for food
Second generation	Crop/fuel chains based on developing conversion technologies	The aim is to switch to lignocellulosic feedstocks
Third generation	Crop/fuel chains based on emerging/future technologies	Includes hydrogen production from biomass or biodegradable waste; also engineered microbes or plants

and slowly depleting resources, and about the greenhouse gas emissions that result from burning fossil fuels.

Alternative energy sources are clearly needed. Plants offer one solution in photosynthesis: the natural energy transformation process that uses sunlight to concentrate atmospheric carbon over 1000-fold to chemical energy in carbohydrate (CH_2O). Plants are primary producers in all food chains and are an irreplaceable resource for feeding the human population; however, throughout history they have also been exploited for fuel, mostly as wood for heating or cooking. Energy derived from coal, oil, natural gas and nuclear power has largely replaced this practice, but now attention is being drawn back to plants as contributors to our future energy mix.

The term 'bioenergy' became fashionable only recently, but energy derived from plants has been pursued before, including during the 1980s when concerns over oil supplies and prices resulted in the use of plant feedstocks for heat and power. Nevertheless, only approx. 46 EJ yr^{-1} (1 EJ = 1^{18} joules) of energy, equating to 13.4% of global primary energy supply, is presently derived from plants (Sims *et al.*, 2006). The total production possible is subject to debate, but between 200 and 400 EJ yr^{-1} (Jurginger *et al.*, 2006) have been proposed, indicating that plants are grossly underexploited.

Recognizing this potential, renewable energy targets from crops have been set by nations. These require that sufficient yields can be sustained from crops. There are many reviews on bioenergy (Hughes & Benemann, 1997; Powlson *et al.*, 2005; Sims *et al.*, 2006; Wright, 2006) and on bioenergy crops (Lewandowski *et al.*, 2003b; Keoleian & Volk, 2005; Samson *et al.*, 2005), but here we focus on what is meant by bioenergy yield. We review yield in some key bioenergy crops, and identify quantity and quality traits. We then address how further sustainable yield improvements can be achieved.

II. Bioenergy, biomass and biofuel crops

Bioenergy crops can be grown for two contrasting markets: power generation (electricity, heat, and combined heat and power) and liquid transport fuels. Clarification of relevant terminology, as used here, is given in Table 1. The terms first, second and third generation are often adopted in relation to biofuels, further details of which are given by, for example, Hamelinck *et al.* (2005); Osowski & Fahlenkamp (2006); Dunnett & Shah (2007). In reality, however, the multiple uses of feedstock confound attempts to classify individual crops into bioenergy types. For example, wheat straw and maize (corn) stovers (first-generation biofuel crops) are also sources of biomass and lignocellulose. Similarly, identifying yield traits only for biofuel crops is difficult, therefore all end uses are covered here.

III. Bioenergy yield traits

Bioenergy yield per unit of land can be defined as the amount of dry matter (DM) or biomass; C available for conversion; or bioenergy (bioethanol, biodiesel, heat/electricity) produced. None should be confused with the C/energy balances, which require energy inputs/outputs and life-cycle analyses. Here we

review contrasting bioenergy crops, focusing on grasses and trees, and identify quantity traits relevant to harvestable DM (t DM ha^{-1} yr^{-1}). Sustainability aspects are covered in Section V.

1. Grain/seed and high-sugar crops

Annual crops, particularly maize and wheat, currently make the largest contribution to bioenergy, particularly biofuels, matched only by perennial sugarcane and oilcrops. Such grain crops have been subject to thousands of years of improvement, but advances during the 'green revolution' (1960–80) were among the most significant.

Wheat (*Triticum* spp.) is a small-grain cereal originating in the fertile crescent of the Middle East. Since earliest agriculture, it has evolved, been selected and more recently been bred into modern hexaploid ($2n = 6x = 42$) bread wheat (*Triticum aestivum*) and the related tetraploid ($2n = 4x = 28$) durum wheat (*Triticum durum*). Wheat straw is already combusted to produce heat and power (for example, at Ely in the UK), and biorefineries are currently under construction that plan to use wheat grain for bioethanol production, at least until other lignocellulosic feedstocks are available.

Most wheat cultivars stand < 1 m tall and tiller freely to produce multiple shoots, which develop almost synchronously. The grain is produced in ears standing above the leaf canopy. Yield is driven primarily by the youngest three leaves on each shoot, with some contribution from the ear. Yield gains have arisen from a combination of genetics and agronomy, and originated from the discovery of short-straw genotypes, especially in Japan (USDA, 2006). The large yield increases of the green revolution were achieved through high levels of application of nitrogen fertilizers (up to 240 kg N ha^{-1} in the UK; MAFF, 2000). Under high N, the short-straw progenitors remained upright, producing greater yields than the long-straw varieties, which were prone to lodging. Production time was also extended as assimilate, no longer partitioned towards stem growth, was used to increase fertile floret number. The dominance of the interaction between DM partitioning, harvest index (HI, the economic yield as a fraction of total aboveground DM) and N fertilizers in wheat breeding before the 1990s can be illustrated by the work of Austin *et al.* (1993). The cultivar Square Head's Master (a common 19th century landrace) and the then modern cv. Brimstone (introduced in 1985) were grown with high N rates, but were supported to prevent lodging. Total aboveground DM was very similar, but with a greater proportion as grain in cv. Brimstone.

In winter wheat cultivars introduced since 1990, increases in aboveground biomass have arisen from greater radiation-use efficiency (RUE) at a constant HI (Calderini *et al.*, 1997; Foulkes *et al.*, 2001; Sylvester-Bradley *et al.*, 2002; Reynolds *et al.*, 2007). The introduction of the 1BL.1RS wheat–rye chromosome translocation contributed to greater pre-anthesis RUE, a greater number of grains per m^2, and greater storage of water-soluble CH_2O in the stem for utilization in grain formation (Foulkes *et al.*, 2007). The grain number increase also potentially contributes to increased post-anthesis RUE by maintaining a strong sink and thereby alleviating feedback inhibition of photosynthesis (Reynolds *et al.*, 2005). One of two wheat–rye translocations (1BL.1RS or 1AL.1RS) is now in almost 25% of new cultivar trial entries in the central USA (Weng *et al.*, 2007). Historically these have been selected for disease resistance, and their effect on absolute yield potential through increased RUE remains to be seen.

Maize (*Zea mays*) ($2n = 2x = 10$) is a large-grain cereal originating in Central America. Limited to the Americas until the 16th century, it has since spread to all tropical, subtropical and many temperate areas of the world. Like wheat, maize is a staple food crop, but in recent years bioethanol production from maize/corn in the USA has risen to volumes equating to, and even exceeding, production from sugarcane in Brazil. Despite being planted on less land than wheat, maize produces greater yields, largely because it utilizes the more efficient C_4 photosynthetic pathway. C_4 plants have biochemical, physiological and morphological adaptations to facilitate CO_2 concentration in the bundle sheath. A critical outcome is a twofold effect on the activity of the large, N-rich enzyme Rubisco. C_4 plants can achieve high rates of photosynthesis with less Rubisco, and therefore less N, than their C_3 counterparts. Although countered to some extent by the N cost of the CO_2-concentrating system (Sage *et al.*, 1987), C_4 plants have greater N-use efficiency (NUE) and water-use efficiency (WUE) compared with C_3 plants such as wheat.

Maize produces a single stem, which can be up to 2 m tall. The female cob is borne approximately half-way up, and the male tassels at the top. This physical separation has simplified the commercial production of F_1 hybrid seed and the exploitation of heterosis. However, sustained yield increases have occurred during the 20th century, suggesting that other factors are also responsible (Tollenaar & Lee, 2002).

Genetic improvements have contributed to 50–60% of overall yield gains in maize (Duvick & Cassman, 1999). A greater number of traits have been modified compared with wheat, although maize has also been selected to resist pests, diseases and lodging. Early, pre-green revolution improvements included reductions in plant height (Duvick & Cassman, 1999), which no doubt improved lodging resistance and DM partitioning towards the grain. However, as maize shows less response to N fertilizer, dwarfing genes were of less benefit than in wheat.

In maize, drought stress and mutual shading tend to increase the time between anthesis and silking (anthesis–silking interval; appearance of a receptive female floral structure) and the appearance of barren spikelets (Lambert & Johnson, 1978; Otegui *et al.*, 1995; Campos *et al.*, 2006). A reduction in the anthesis–silking interval resulted in greater drought

Fig. 1 Switchgrass (2 m high) growing on Rothamsted (UK) field plots.

tolerance in modern varieties. Moreover, reductions in anthesis–silking interval, coupled with more vertical leaf angles, allowed cultivation of two- to threefold more plants per unit area (Lambert & Johnson, 1978; Duvick & Cassman, 1999; Uribelarrea *et al.*, 2002). Over the past 40 yr, most of the genetic yield gain has arisen from traits associated with tolerance to greater densities (Duvick & Cassman, 1999). The effects of grain yield improvement on total DM yield and HI are less clear. Russell (1985) and Luquet *et al.* (2006) reported increases in DM yield and HI, probably because of the strong sink created by the increased number of developing grains, but Tollenaar (1989) found no significant changes.

Sugarcane (*Saccharum officinarum*) is a large (> 2 m tall) perennial C_4 grass, originating mostly in South-East Asia. Modern cultivars are often interspecific hybrids resulting from breeding programmes, and have spread all around the tropics and subtropics. Sugarcane is one of the most productive terrestrial plants, and yields of 80 t DM ha^{-1} in a 12-month growing season have been reported on Hawaii (Elawad *et al.*, 1980). However, it is propagated from stem cuttings, and yield declines yearly following planting mainly because of root pathogen build-up (Magarey *et al.*, 1997) and the depletion of soil minerals. As a result, crops are frequently removed after 5 yr. Late-season millable stem death can limit maximum yield (Robertson *et al.*, 1996), a phenomenon that may be partially explained by yield decline (Bell & Garside, 2005).

Sugarcane stems contain 20% sucrose, which has to be processed quickly after harvesting for maximum extraction. The RUE of intensively managed sugarcane is very high, and DM partitioning towards sucrose in the stem (increased HI) may be a more suitable breeding goal than increased biomass (Inman-Bamber *et al.*, 2002). However, the long history of stable HI suggests that this may be a conservative trait (Muchow *et al.*, 1996). Alternatively, high sucrose concentration may be strongly associated with low cane yield, resulting in selection against high sucrose in breeding programmes in the past (Jackson, 2005).

The association of sugarcane with endophytic diazotrophic bacteria results in an enhanced N supply to the plant, probably through utilization of soil N rather than fixation of atmospheric N_2 (Andrews *et al.*, 2003). In Brazil, the historical use of relatively low N fertilizer rates may have selected for genotypes with high proportions of fixed N. Boddey *et al.* (2001) and Baldani *et al.* (2002) estimate that 25–60% of the N requirement is provided this way. However, across the world, N application rates vary (100–275 kg N ha^{-1}), with high rates in Florida, where leaching and denitrification are potentially large (Rice *et al.*, 2007); in southern India the exceptionally large rate of 275 kg N ha^{-1} could be reduced by inoculation with diazotrophic bacteria (Muthukumarasamy *et al.*, 1999).

2. Perennial rhizomatous grasses

Several rhizomatous grasses are grown worldwide for bioenergy (Lewandowski *et al.*, 2003b); the focus here is on the most advanced and widely used, switchgrass and *Miscanthus*, which utilize the C_4 photosynthetic pathway.

Switchgrass (*Panicum virgatum*) (Fig. 1) is a warm-season grass belonging to the Panicoidea. It can reach 3 m height, and comprises two ecotypes. The upland ecotypes are usually octoploids ($2n = 8x = 72$) or occasionally hexaploids ($2n = 6x = 54$) and are shorter, fine-stemmed, and more adapted to drier habitats. The lowland ecotypes are usually tetraploid ($2n = 4x = 36$), more resistant to rust, coarse-stemmed, tall-growing, more bunchy in growth and adapted to wetter sites. They require a longer growing season as they mature later than upland ecotypes (Lewandowski *et al.*, 2003b).

Switchgrass is a popular bioenergy crop in the USA as it is a native species, shows a wide geographical distribution from 55°N latitude to central Mexico, is established from seed, maintains high productivity across its wide range, can easily be integrated into conventional farming, and has the flexibility of also being a forage crop (Lewandowski *et al.*, 2003b). Switchgrass breeding began in the USA in the 1930s for fodder production. The DM yield increases of many cultivars have resulted from selections on natural populations. Over 10 yr, the US Department of Energy achieved yield increases of approx. 50% through selecting the best regionally adapted varieties, optimizing cutting frequency and timing, and reducing the level and timing of N fertilization (McLaughlin & Walsh, 1998; Lewandowski *et al.*, 2003b; McLaughlin & Adams Kszos, 2005).

Switchgrass productivity is limited predominantly by N and water. Nitrogen content aboveground is highest mid-season, but as the plants senesce, N is translocated to the crown/rhizomes and roots. Once mature, switchgrass grows as a closed canopy and allocates a large fraction of the photosynthetic products to maintenance of the large, active root system.

Fig. 2 *Miscanthus* × *giganteus* (3.5 m high) growing at Rothamsted (UK).

This high investment diverts C to belowground biomass, and only 33–66% of maximum production capacity is typical during the first 2 yr of growth. Recycling also results in a lower requirement for fertilizers (McLaughlin & Walsh, 1998). Trials indicated that response to nutrients is site-specific. Typically, no applications of P and K are required, and 50 kg ha^{-1} N is sufficient for many single-cut systems. This finding significantly reduced production costs, halving N fertilization from what was originally thought necessary (McLaughlin & Adams Kszos, 2005).

There is significant potential for further yield increase in switchgrass, as considerable heritable variation exists for DM yield and related physiological traits (Kiniry *et al.*, 1999; McLaughlin *et al.*, 2006). Highly significant differences have been reported in photosynthesis, stomatal conductance, transpiration and WUE, but it is the balance of C assimilated per unit of water transpired that is most closely linked to high biomass yield (McLaughlin & Adams Kszos, 2005).

Miscanthus Originates from East-Asia. The most important species for bioenergy are *Miscanthus sacchariflorus* ($2n = 4x = 76$), *Miscanthus sinensis* ($2n = 2x = 38$), and particularly the sterile triploid hybrid between these, *Miscanthus* × *giganteus* ($2n = 3x = 57$) (Fig. 2). *Miscanthus sacchariflorus* is more adapted to warmer climates and has a broad creeping rhizome and thicker stems, while *M. sinensis* is more winter-hardy and has tuft-forming rhizomes with thinner stems. The original *M.* × *giganteus* hybrid, which arose from a rare natural hybridization, has intermediate rhizome and stem characteristics, and is more vigorous and higher-yielding (Lewandowski *et al.*, 2003b).

Miscanthus × *giganteus* begins growth from the dormant winter rhizome when soil temperatures reach approx. 9°C, and leaf expansion occurs between 5 and 10°C (Lewandowski *et al.*, 2003b). In temperate regions, this limits productivity as the early spring radiation is missed by late emergence. However, for earlier emergence, shoots would need to be tolerant of frost. Farrell *et al.* (2006) found that *M. sinesis* clone Sin-H9 had lower thermal requirements and higher frost tolerance, suggesting that yield gains in temperate zones could be achieved if these traits were combined with the high shoot density, RUE and rapid leaf extension of the best *M.* × *giganteus* clones.

M. × *giganteus* is sterile and can only be propagated as rhizomes or by tissue culture. It generally needs 3–5 yr growth before it is yielding maturely. The crop is typically harvested when the stems are fully dried out. Although biomass yield drops by 25% during the drying period, this enables nutrients to be remobilized to the rhizome. In European trials, genotypic variation in plant height was associated with flowering time (the tallest genotypes flowering later). Late flowering was associated with late senescence, and the late-flowering, late-senescing genotypes gave higher yields. However, this relationship did not hold in more northerly regions, probably because autumn frosts killed the leaves, reducing translocation of nutrients to the rhizome (Clifton-Brown *et al.*, 2001). The 'mother rhizome' provides assimilate for new shoot production, exhausting as the year progresses (Midorikawa *et al.*, 1975). Assimilates produced from photosynthesis accumulate in the new daughter rhizome, doubling its weight by the latter part of the season. By the winter, most N remains in the roots, rhizomes and litter (Christian *et al.*, 1997, 2006). Nutrient recycling is normally highly efficient, and generally N inputs are minimal. However, data on N response are variable – a finding most certainly related to both genotypic variation and differences in the fertility of sites (Christian *et al.*, 1997, 2006; Strasil, 1999; Heaton *et al.*, 2004; Lewandowski & Schmidt, 2006; Danalatos *et al.*, 2007).

As a C_4 plant, *Miscanthus* has a high WUE, however, substantial amounts of water are needed to sustain maximum growth. Pot experiments indicated that *M.* × *giganteus* and *M. sacchariflorus* responded to water stress by senescing and losing leaf area, while *M. sinensis* reduced its leaf conductance and was able to remain green even when water was severely limiting. The WUE did not differ significantly between genotypes or treatments, but in water-stressed *M.* × *giganteus* and *M. sacchariflorus*, root growth constituted the greater portion of the total biomass gain, while rhizome growth was proportionally greater in *M. sinensis* (Clifton-Brown & Lewandowski, 2000).

3. Fast-growing trees

Poplars (*Populus*) and willows (*Salix*) constitute the family Salicaeae. Their basic chromosome number is 19 and, particularly in willow, many ploidies exist. They undergo C_3 photosynthesis and have among the highest CO_2-exchange rates, light-use efficiencies, and photosynthetic capacities of woody species, with values well within the range of most C_3 agricultural plants (Raven, 1992; Ceulemans & Isebrands,

Fig. 3 Short-rotation coppice (SRC) willow at Rothamsted (UK) being assessed for stem traits.

1996; Ceulemans *et al.*, 1996). Fast-growing poplars and willows can be cultivated in short-rotation forestry cycles of 15–18 yr, but in short-rotation coppice (SRC) this is reduced further by cut-back/coppicing at 3–5-yr intervals. Coppicing reinvigorates plants, accelerating growth towards a theoretical maximum which may contribute to high yields (Tschanplinski & Blake, 1989; Sennerby-Forsse, 1995).

Willows Of the 330–500 species (Argus, 1997) of willow, the shrub willows (*Salix viminalis* in Europe and *Salix eriocephala* in North America and Canada) are deemed most suitable as bioenergy crops (Stott, 1984; Larsson, 1998; Kuzovkina *et al.*, 2008). Other species used include *S. dasyclados*, *S. schwerinii*, *S. triandra*, *S. caprea*, *S. daphnoides* and *S. purpurea*, and many varieties are interspecific hybrids.

Coppicing has been a traditional practice in willow. It removes apical dominance, allowing proleptic shoot development from otherwise dormant axillary buds on the stool. Willows vary in coppicing response, with the number of resprouting shoots correlated with the number and behaviour of buds (Sennerby-Forsse & Zsuffa, 1995). In *S. viminalis*, the main shoot primordia develop first, followed by two lateral primordia, which give rise to weaker, shorter shoots that contribute to fast development of the canopy, but are later suppressed. Thus coppiced willow (Fig. 3) is characterized by fast growth of many stems, followed by progressive self-thinning (Sennerby-Forsse, 1995; Ross & Ross, 1998; Sannervik *et al.*, 2006). Adventitious primordia can also grow out in the year when they are formed to produce sylleptic shoots, but these do not contribute to yield (Sennerby-Forsse, 1995; Ronnberg-Wastljung & Gullberg, 1999). In the second and third years (when there is no cutback/coppicing), sprouting occurs from the apex of first- and second-year stems, respectively (Ross & Ross, 1998). *Salix viminalis* has narrow leaves that are 15 times longer than they are wide. Plantations are characterized by a large number of leaves per stem and great variability of leaf area (Ross & Ross, 1998). Between 30 and 50 leaves are present in the expanding leaf zone, compared with five to 10 in poplar, and the leaf area index (LAI) reaches maximum values near midsummer (Isebrands *et al.*, 1996).

Yield increases in willows to date have been achieved by selecting for stem characteristics (height, diameter, straightness) and coppicing response (number of shoots, shoot vigour), as well as resistance to diseases, insects and frost damage (Stott, 1984; Vihera-Aarnio, 1988; Larsson, 1998). When compared with a low-yielding line (L78183), the high-yielding cv. Tora has a lower LAI (Robinson *et al.*, 2004) and maintains a high level of light interception throughout the canopy depth (Weih & Ronnberg-Wastljung, 2007). However, among 32 willows at least two alternative growth strategies were identified: either a large number of thin stems (typically 11 per stool), relatively low LAI and specific leaf area (SLA); or fewer, larger-diameter stems (typically six per stool), and high LAI and SLA. Both give high yield; multiple ideotypes may need to be selected (Tharakan *et al.*, 2005).

Fast growth early in the season in response to lapse of thermal time is of key importance (Cannell *et al.*, 1987). In *S. viminalis*, starting bud flush earlier (even by a few days) has a greater influence on total stem weight than delaying growth cessation in autumn (Ronnberg-Wastljung & Gullberg, 1999). However, cold tolerance is needed as the risk of frost damage is increased, particularly in northern zones (Ronnberg-Wastljung & Gullberg, 1999; Ronnberg-Wastljung, 2001; Tsarouhas *et al.*, 2003).

In *S. viminalis*, immediately after breaking dormancy, C stored in the stems is allocated to developing new roots and leaves. Newly assimilated C is also allocated in this way, but this drops to 15% in the second month as C is allocated to the secondary growth of the stems (de Neergaard *et al.*, 2002). As the stems grow and their leaves mature, they become independent of root reserves, and eventually the C flux is reversed and C is exported from the leaves (Ceulemans *et al.*, 1996; Isebrands *et al.*, 1996). Nitrogen is remobilized during the perennial cycle, and once canopy growth is complete, a significant part of the annual nutrient demands are met by efficient internal cycling and reabsorption from leaf litter. The long-term nutrient requirements in willow are restricted to the amounts lost at harvest, with between 30 and 80 kg N required (Sennerby-Forsse, 1995).

The efficient mobilization of resources necessary for spring growth results from the reactivation of mature overwintered vessels (Lawton, 1976; Sennerby-Forsse, 1986). *Salix* spp. are diffuse-porous trees with heterocellular rays, and several years of xylem become simultaneously functional. The resumption of flow is marked by a flux of concentrated sugars, resulting from mobilization of CH_2O in the parenchyma cells of xylem rays in the roots (Raven, 1992). Willow root biomass may be equal to, or greater than, the total aboveground biomass (Porter *et al.*, 1993).

To avoid interstool competition and to gain optimal productivity, planting densities of 15 000–18 000 stools ha^{-1} and a rotation cycle of 3 yr are widely adopted (Ledin, 1996; Verwijst, 1996a, 1996b; Kopp *et al.*, 1997). Higher densities (20 000–25 000) have been reported for new varieties (Wilkinson *et al.*, 1999), but if densities are too high, or coppicing cycles are shortened, excessive self-thinning and stool mortality occur, reducing yields significantly (Verwijst, 1996a, 1996b; Kopp *et al.*, 1997).

Simultaneous development of many shoots per stool, leading to a rapid build-up of large leaf area and early canopy closure, is an effective strategy for biomass accumulation (Sennerby-Forsse, 1995), but it can result in high transpiration. Weih & Nordh (2002) and Linderson *et al.* (2007) found that intrinsic WUE and relative water content varied among willow genotypes, and when water is strongly limiting, clones with higher intrinsic WUE produced higher shoot biomass. Carbon partitioning between roots and shoots is also sensitive to water availability, enabling drought-tolerant willows to withstand dry periods without severe reduction in DM yield (Lindroth *et al.*, 1994).

Poplars Compared with willows, there are relatively few poplar species that fall into six morphologically and ecologically distinct sections. Of these, *Aigeiros* (cottonwoods, *Populus nigra*) and *Populus* (aspens, white poplars) are of most relevance for bioenergy.

Traditional breeding of poplars as single-trunk trees for wood production in short-rotation forestry has been extremely successful. Hybrid poplars, in particular, are faster growing and more productive, with larger LAI and longer leaf area duration than parental species under short-rotation forestry regimes. Superior poplar clones also have a low canopy extinction coefficient (k), leading to good distribution of solar radiation to depth in the canopy, and high rates of whole-canopy photosynthesis (Heilman *et al.*, 1996).

Traits important for DM yield in poplar, as summarized by Dickmann *et al.* (2001), are similar to those for willow. However, sylleptic branches contribute positively to biomass in poplar by creating additional leaf area. They also export a larger proportion of their fixed C to the stem, compared with proleptic branches, resulting in higher stem diameter growth rates (Scarascia-Mugnozza *et al.*, 1999) and stem volumes (Ceulemans *et al.*, 1990; DeBell *et al.*, 1996). Carbon is transported in the phloem as simple sugars (primarily sucrose) (Dickmann *et al.*, 2001), with developing leaves transporting C to other younger leaves and the apex, and fully mature leaves transporting C predominantly downwards to stems and roots (Isebrands *et al.*, 1996).

Poplars were not traditionally selected for growing as coppice, but respond to coppicing similarly to willows, initially producing many shoots that are subsequently thinned (Laureysens *et al.*, 2003). Competition for light can result in very high shoot and stool mortality, particularly in the first year, and poplars are usually grown at lower densities (e.g. 10 000 cuttings ha^{-1}; DeBell *et al.*, 1996; Armstrong *et al.*, 1999; Ceulemans & Deraedt, 1999). Some clones take longer to reach maximum growth rates, and although 3–4-yr cycles are successful (Deckmyn *et al.*, 2004), 6–7 yr for some poplars and 10–12 yr for aspen have been recommended (Kauter *et al.*, 2003).

In comparative short-rotation forestry and SRC trials, coppiced systems yielded less, although coppicing enhanced intrinsic growth rates (Herve & Ceulemans, 1996; Proe *et al.*, 2002). However, results from a larger range of 17 poplar varieties at Boom, Belgium revealed variability in coppicing response, shoot and stool mortality rates, and yield. Similarly to willow, high biomass was achievable from plants with contrasting growth strategies (Laureysens *et al.*, 2005). More detailed measurements of the 17 genotypes revealed considerable variation in several leaf and petiole traits (Al Afas *et al.*, 2005). However, unlike previous reports (Taylor *et al.*, 2001), leaf size was not found to be a good indicator of high biomass productivity.

More recent studies of two poplar families in Italy and France showed that stem volume and stem volume growth rate were closely linked to the leaf area of the largest leaf, and to the length and dry weight of its petiole. Other traits, such as SLA and leaf N content, were dependent on site or family (Marron *et al.*, 2007). Tradeoffs between physiological and morphological traits have been discussed previously (Weih, 2003). The relative importance of different growth variables may be difficult to define because of the numerous interactions that occur between the environment and internal plant processes (Karacic & Weih, 2006).

Biomass yields in the Boom trial during the establishment year were low, probably because of investment in root growth (Deraedt & Ceulemans, 1998). Poplar and willow roots share similarities, although poplars usually develop thicker, longer tap roots and have larger stem and maximum root diameters (Crow & Houston, 2004). Remobilization of resources is a crucial aspect of the perennial cycle in poplar, where it has been well studied (Cooke & Weih, 2005). Nitrogen is stored in bark-storage proteins in the parenchyma cells of stems and roots during winter. In advance of bud burst, the N reserves are broken down and amino acids are transported to supply N for the expanding buds. The root system of poplar also represents a large portion of the tree's C economy, with seasonal loading of CH_2O as well as nutrients, and has been shown to be important in water-stress resistance.

Biomass yield in poplar can be severely compromised when water is limiting (Liang *et al.*, 2006), and studies indicate that poplar is less responsive than willow to water stress and to changes in atmospheric vapour pressure (Hinckley *et al.*, 1994; Johnson *et al.*, 2002). However, poplar clones and species differ in the way they react to water stress conditions, suggesting that it may be possible to identify genotypes with improved response (Bungart *et al.*, 2001; Street *et al.*, 2006).

Table 2 Consensus values for quality attributes of the energy crops discussed here

	Moisture	Ash	Volatiles	Acid detergent lignin	Hemi-cellulose	Cellulose	Starch	Sucrose	Total carbohydrate	Oil	CV MJ kg^{-1}	Density t m^{-3}	N	K	Na	Cl	Si	S
Wheat grain	14	2.0		3	3	2	70	3	82	< 2		0.7–0.8	2.5					
Wheat straw	16	6.2	63.5	15.1	24.6	33.2					18.02	0.35	0.6	1.9		0.4		0.31
Maize grain	10	1.6					74		84	5		0.8	2.3	0.42				
Maize stover	20	5.1	80.9	10.4	28	35					18.1					1.48		0.05
Sugarcane	70			7	8	24		47										
Bagasse						48												
Switchgrass	20	3.7	71.5	6.1	36	31.6					17.9	0.45	0.6			0.07		0.04
Miscanthus	20	2.0	82.1	10.5	15.9	57.6					18.2	0.35	0.37	0.41	0.03	0.39	1.0	0.04
Willow	25	1.3		19	14.0	55.9					19.3	0.22	0.4	0.16	0.01	0.24	0.18	0.03
Poplar	25	1.8	77.9	20	23	40					19.2	0.27	0.6	0.37	0.004	0.008		0.02
Coal (Daw Mills)	7	6.2	34.1								31.06	0.8	1.1	0.22	0.08	0.24	1.8	1.5

Values vary with site, season, cultivar and management. Values are from one or more of: Legendre & Burner (1995); Ledin (1996); Muchow *et al*. (1996); Christian & Riche (1999); Szczukowski *et al*. (2002); Lewandowski *et al*. (2003a); Tharakan *et al*. (2003); Worldwatch Institute (2006); Bridgeman *et al*. (2007); Phyllis (2007); P. R. Shewry (pers. comm.); authors' own unpublished data. All values are percentages of mass at 100% DM, except moisture (% as received), CV (MJ kg^{-1} DM) and density (t m^{-3} as received).

IV. Bioenergy composition traits

The composition of plant DM varies considerably among bioenergy crops (Table 2), and this has significance for conversion to energy and thus for bioenergy yields.

Bioenergy conversion technologies divide into two broad categories (biological and thermal), and the quality criteria most suited to each are quite distinct. Biological conversion processes, fermentation and anaerobic digestion, operate most efficiently when presented with simple carbohydrates (sucrose and starch) that are readily accessible and broken down by enzymatic systems. Therefore sugarcane and cereal grains are the current preferred feedstock. The moisture content is not important, except in relation to storage, nor is the inorganic fraction, which remains in the residue and may be utilized for animal feed or fertilizer. The complicating factor may be if the residue is to be used as an energy source via thermal technologies. Alkali metals (e.g. K and Na) in the feedstock for combustion or gasification can cause problems with slagging and fouling, whereby ash quality falls and boiler tubes may be coated in harmful deposits, or chemically eroded. The majority of N- or S-containing compounds in the feedstock form NO_x or SO_x in the exhaust gasses, thereby contributing to greenhouse gas emissions or acidification, respectively. Therefore thermal technologies tend to source switchgrass, miscanthus, willow or poplar (or other wood sources), which are all materials low in simple carbohydrates and inorganic fraction. Unlike biological conversion, moisture content is an important consideration beyond crop storage in thermal conversion technologies, as the calorific value is negatively correlated with moisture content. Pyrolysis is a slightly unusual case: it is a thermal energy conversion where large lignin fractions in the feedstock can reduce 'bio-oil' yield and quality.

Biofuel production from sugars, starches and oils of food crops utilize well developed conversion processes. However, although they are instrumental in growing the market, it is generally accepted that biofuels produced in these ways are not long-term solutions (see Section V). Increasing emphasis is being placed on rendering more complex carbohydrates, particularly cellulose present in plant cell walls, accessible to enzymatic breakdown. Cellulose is the most abundant biopolymer on Earth, and could provide a potentially vast source of feedstock for biofuels. However, the principal barrier to processing biofuels in this way is the recalcitrance of lignocellulose to biological and chemical degradation. The plant cell wall is a dynamic composite network of complex polymers of four major types: hemicellulose, pectin, cellulose and lignin (Joshi & Mansfield, 2007). Lignin and phenolic acid esters render the cellulose less accessible through cross-linkages. Cell wall structure differs between the trees and perennial grasses, the former generally containing higher amounts of lignin (Table 2) and the latter more abundant phenolic acids such as ferulic and *p*-coumaric acids (Akin, 2007). The genetic and

Table 3 Framework of principles for evaluating the sustainability of bioenergy production systems

Environmental principles	Social principles
Greenhouse gas balance (life-cycle basis)	Compliance with applicable law
Maintenance of above- and belowground carbon stocks	Protection of rights for contractors and subcontractors
Conservation of biodiversity	Freedom of association and right to collective bargaining
Sustainable water use	Reasonable working hours
Maintenance of soil fertility	Nonexploitation of child labour
	Application of proper health and safety
	Wages and compensation
	Discrimination
	Forced labour
	Land rights issues

biochemical regulation of cellulose biosynthesis remains to be resolved, and the dynamic complexity of cell wall composition presents considerable challenges (Rose *et al.*, 2004; Joshi & Mansfield, 2007). A massive research push is currently in motion to improve understanding of cell wall structure, assembly, disassembly and dynamics (USDA, 2005), and to explore chemistries and technologies to overcome the barrier of lignocellulsic recalcitrance (Hamelinck *et al.*, 2005; Dunnett & Shah, 2007; Lange, 2007). A much greater feedstock range would then be suitable for biological conversion, including the perennial grasses and trees, as well as other materials such as straw and stovers.

V. Sustainable bioenergy production from crops

The sustainability of bioenergy chains is the subject for a review in its own right. Here we focus on issues relating to bioenergy yield.

Systems-modelling tools allow the feedstock production (crop-related) segments of a bioenergy chain to be developed and assessed within the context of the whole chain. A balanced approach can then be undertaken to understand and predict environmental, social and economic impacts of specific feedstocks (both production and use). Sustainability monitoring is then carried out using criteria and indicators to measure the performance under each of 15 generic principles covering the environmental and social components (Table 3). Economic performance is considered to be accounted for by the economic viability of each supply chain under the policy and regulatory environments of each country. There are a number of indicators to which the sustainability performance is most sensitive with regard to feedstock production. These suggest that to improve sustainability performance, emphasis should be placed on increasing yields per unit input (including solar radiation, nutrients – particularly N, and water); minimizing emissions to air, water and soil; and developing crop production systems that maintain or improve biodiversity.

As described in Section III, yields of annual crops are highly dependent on high N inputs, and on the use of varieties with shorter stems and increased partitioning to the harvestable seed/grain. By contrast, high-yielding perennial grasses and trees achieve impressive stem growth and high DM in vegetative parts, with minimal N fertilizer (20–50% less, allowing for site-specific variation), due to efficient remobilization of reserves. This difference has a large impact on life-cycle analyses of bioenergy chains for the different crops, because N fertilizers are energy-intensive to make.

Life-cycle analysis of bioenergy chains is a highly complex and sometimes controversial science. Results vary depending on the boundaries assigned to the chain, as well on the assumptions behind some of the calculations used (Rafaschieri *et al.*, 1999; Heller *et al.*, 2003; Heller *et al.*, 2004; Keoleian & Volk, 2005; Sims *et al.*, 2006; von Blottnitz & Curran, 2007). Nevertheless, in general, life-cycle analyses indicate that the energy savings and greenhouse gas reductions achieved for annual crops are, at worst, low or even negative and, at best, not as high as those for perennial bioenergy crops (Heller *et al.*, 2003; Cocco, 2007). Recent estimates for biofuel production from switchgrass indicate 94% greenhouse gas reductions compared with gasoline, and 540% more renewable than nonrenewable energy consumed (Schmer *et al.*, 2008). Carbon sequestration possibilities are also higher for the perennial crops (Lemus & Lal, 2005; Sartori *et al.*, 2006).

The association of crops such as sugarcane with N-fixing bacteria enhances N supply to the plant, thus potentially contributing an important saving to the greenhouse gas (and financial) budgets of growing this crop. Associations with microrganisms are known for perennial bioenergy crops (Kirchhof *et al.*, 1997, 2001; Baum *et al.*, 2002; Paradi & Barr, 2006), but their contribution to the overal N budget still needs to be established. Outside the Leguminosae, however, there appear to be few crops that are able to fix N. It may also be possible to intercrop the energy crop with a legume, for example *Miscanthus* with hairy vetch (Miguez, 2007).

One of the largest threats to sustainable production from perennial crops is yield losses caused by pests or diseases. The agrochemical control applied to food crops is uneconomical, would increase inputs, and is difficult in practice once the

Fig. 4 *Miscanthus* × *giganteus* growing in the English countryside.

crops are well established. Durable resistance is the only crop protection route possible. Pests and pathogens do not appear to cause major problems in the perennial grasses outside the tropics, although this may change with future expansion of the crops. However, in SRC diseases, especially rust (*Melamposora* spp.), can reduce yields by 40% (Pei *et al.*, 1997). Rust was the cause of major crop failure in poplars grown in Europe, and in willows several older varieties are now susceptible. Up to seven species of *Melampsora* have been identified on willows in the UK alone (Pei *et al.*, 1993), of which *Melampsora epitea* is the most important on SRC. Fourteen distinct pathotypes of *M. epitea* var. *epitea* have been identified (Pei *et al.*, 1997). Fortunately, the diversity of willows provides a rich source of species with new resistances, which have been bred into many new varieties. To date, this strategy has been very successful, particularly utilizing species from Siberia for crossing with *S. viminalis*. Moreover, studies have shown that the use of clonal mixtures in SRC plantations can be highly effective in reducing the impact of rust disease (McCracken & Dawson, 1997, 2003; Hunter *et al.*, 2002).

Prominent among other considerations of sustainability (Granda *et al.*, 2007) are concerns that using food crops for bioenergy directly competes with food production (Cassman & Liska, 2007). This is not entirely reprieved for perennial (nonfood) bioenergy crops, as competition over land remains; however, the argument presented is that, because they are so efficient at recycling nutrients, they can be grown on marginal land.

Perennial bioenergy crops also differ in a number of physical traits and are managed in ways quite different from arable crops. In particular, perennial bioenergy crops will remain in place for a long time (approx. 25 yr anticipated for SRC; approx. 20 yr for *Miscanthus*), harvest is normally in winter/early spring, they are deep-rooting, generally high water-users, and also very tall (3–5 m). These factors modify the appearance of the landscape (Fig. 4) and have potential implications for hydrology and biodiversity. The environmental impacts of large-scale conversion of land from arable cropping to perennial bioenergy crops need to be considered, and are currently the subject of much research. Results so far suggest that many positive benefits may accrue, but this depends on the specific bioenergy crops, the existing land use, the scale of planting, and the management practices applied (Abrahamson *et al.*, 1998; Volk *et al.*, 2004; Rowe *et al.*, 2007).

VI. Increasing bioenergy yields in a sustainable way

Several authors have discussed optimal traits for bioenergy production in specific crops (Dickmann *et al.* 2001; Ragauskas *et al.* 2006; Torney *et al.*, 2007), and the concept of crop ideotypes (Donald, 1968) has been applied to individual bioenergy crops, particularly maize and poplar (Dickmann *et al.*, 2001; Ragauskas *et al.*, 2006). Here we review bioenergy quantity and quality traits in a range of different crops, from which (focusing particularly on the perennial grasses and trees) target traits for future sustainable yield and quality improvement can be identified (Table 4).

Improvement of these traits could be achieved through two basic routes. The more conventional makes use of existing variation (or mutagenesis-derived variation) for crossing and selection programmes, but can be enhanced through improved knowledge of the genetic basis of the traits and the identification of molecular markers for marker-assisted selection (Price, 2006; Sorrells, 2007). The alternative approach utilizes transgenic or genetic modification (GM) technologies to introduce new genes, modify existing genes, or interfere with gene expression (Torney *et al.*, 2007). For both routes, advances in molecular mapping, whole-genome sequencing, 'omics' (transcriptomics, proteomics, metabolomics), whole-genome scans and bioinformatics provide powerful approaches for gene discovery (Brunner *et al.*, 2007). However, identifying whether traits are determined by major genes or quantitative trait loci (QTL) is of foremost importance.

Worldwide, there has been huge effort aimed at identifying the QTL and genes underlying important yield and composition traits in the major food crops (e.g. Sorrells, 2007). Although the resources allocated to perennial bioenergy crops have been limited to date, significant advances have been made. In particular, poplar has been developed as a model tree, and knowledge of its biology, physiology, genetics and genomics arguably places it as the most advanced of all perennial bioenergy crops (Taylor, 2002; Wulschleger *et al.*, 2002; Tuskan *et al.*, 2006). The development of a first-draft whole-genome sequence, and associated 'omic' resources (Tuskan *et al.*, 2006), has significantly enhanced gene discovery in poplar and willow, due to the collinearity of their genomes (Hanley *et al.*, 2006). European and US efforts in poplar have led to the identification of QTL for many of the traits in Table 3 (Taylor

Table 4 Suggested traits for sustainable yield and quality improvement

Quantity traits	Quality traits
Maximizing radiation interception	**Ease of harvesting/storage**
Early bud flush/spring growth	Straight, upright stems
Frost (cold) tolerance	Resistance to lodging
Fast canopy closure	Low moisture content
Tolerance of high plant density	Disease/microbial breakdown resistance postharvest
Resistance to lodging	Low dust
Maximizing radiation use efficiency	**Suitability for thermal conversion technologies**
Low-temperature-tolerant C_4 photosynthesis	Maximum energy density
Efficient C_3 and C_4 photosynthetic rates	Optimal flowering and senescence (for remobilization)
Canopies with low extinction coefficients	Efficient nutrient recycling
Leaf traits for efficient light capture	
High nitrogen-use efficiency	**Suitability for biological conversion technologies**
Drought tolerance	Improved accessibility of carbon in the cell wall for industrial processing
Disease resistance (including microbial breakdown postsenescence)	Maximum density/high proportion of 'available' energy substrates
Pest resistance	*Desirable:* optimal flowering and senescence (for remobilization)
Resistance to lodging	*Desirable:* efficient nutrient recycling
Maximizing water-use efficiency	**Health and safety**
Rapid attainment of maximum growth rate (drought avoidance)	Disease/microbial breakdown resistance postharvest
Drought stress tolerance	Low dust
Environmental and financial sustainability	
Optimal flowering and senescence (for remobilization)	
Efficient nutrient recycling	
Optimal root/shoot partitioning	
High nitrogen-use efficiency	

et al., 2001; Bunn *et al.*, 2004; Street *et al.*, 2006; Rae *et al.*, 2007), and approaches such as array analyses are leading to the identification of candidate genes. Similarly, genetic maps have been published for willow, and QTL and candidate genes have been identified (Ronnberg-Wastljung *et al.*, 2005, 2006; Hanley *et al.*, 2006). Considerable progress has been made in understanding pathogen dynamics and host–pathogen relations (Pei *et al.*, 2002, 2003, 2004, 2005). Similar efforts have been directed towards insect resistance, including identification of leaf volatiles and secondary metabolites that act as attractants/repellents (Glynn *et al.*, 2004; Nordman *et al.*, 2005; Ronnberg-Wastljung *et al.*, 2006).

Identification of genetic determinants in the perennial grasses is made difficult because of the polyploid nature of the commercial crops. However, genetic maps of *M. sinensis* have been developed (Atienza *et al.*, 2002) and QTL have been identified (Atienza *et al.*, 2003). Knowledge and markers from other Gramineae, for example maize (Hernandez *et al.*, 2001) and, more recently, sugarcane and *Brachypodium*, coupled with the current research investment going into these crops, is accelerating progress.

Six potential routes for increasing biomass by improving photosynthetic efficiency have been reviewed (Long *et al.*, 2006). As described earlier (Section III), C_4 plants have particular advantages for productivity (Long, 1983; Beale *et al.*, 1999); however, in cooler regions C_3 plants may be equally, if not more, productive. Modifying C_4 photosynthesis so that temperature is not limiting may be a more reachable target than trying to covert a C_3 into a C_4 (Beale & Long, 1995; Beale *et al.*, 1996).

Target genes for biomass yield and quality improvement through GM approaches have been identified previously (Ragauskas *et al.*, 2006; Sims *et al.*, 2006). Torney *et al.* (2007) recently reviewed GM approaches in maize, and illustrate possible gene targets for improving bioethanol production from kernels (mainly starch) and stovers (mainly lignocellulose). These include modification of starch composition to render the crystals more digestible by enzymes, or to reduce the energy requirements for the starch-to-ethanol conversion, and modification of cell wall composition so that the lignocellulose complex is more accessible to cellulases. Approaches to improve biomass yields, reviewed by Torney, include genes involved in stress tolerance, photosynthesis and sink strength. Similarly, Sticklen (2006) reviewed GM approaches to improving biomass characteristics for biofuel production from crops such as poplar, focusing on manipulation of plants to alter lignin content; self-produce cellulase enzymes for cellulose degradation and lignase enzymes for lignin degradation; and increase biomass production, for example by delaying flowering, thereby diverting energy normally used for reproduction into

Table 5 Plant growth models for various bioenergy crops

Plant type	Crop type	Species	Model	Complexity	Phenotypic information	Reference
Arable	Cereals (C_3, C_4)	Wheat, maize Oilseed rape	CERES	Physiology/physics-based, sources and sinks, no interaction	Phenology (leaf appearance) partitioning	Ritchie & Otter (1985); Brooking (1993); Gabrielle *et al.* (1998)
	Beet tuber		SUCROS/Wofost	Physiology-based, photosynthesis source limited	Phenology, partitioning tables	van Ittersum *et al.* (2003)
Grassland	C_3–humid–temperate	Ryegrass, Timothy	LINGRA	Physiology-based, RUE Michaelis–Menten, sink–source interaction	Phenology (vegetative, reproductive), morphology, density	Schapendonk *et al.* (1998); Rodriguez *et al.* (1999)
	C_4–humid–warm	Switchgrass Miscanthus	ALMANAC Noname MISCANMOD	Physiology-based, RUE RUE Physiology-based, RUE	Phenology, potential LAI, rooting depth Thermal requirement, frost resistance Leaf expansion	Kiniry *et al.* (1996) Clifton-Brown & Jones (1997); Clifton-Brown *et al.* (2004); Price *et al.* (2004)
Woody	Forest trees	Eucalyptus	SILVA	RUE, potential and water-limited growth	LAI dynamics, harvest index, reference crop	van den Broek *et al.* (2001)
	Short-rotation coppice	Willow	Noname	Empirical	Water use efficiency, allocation	Lindroth & Bath (1999)
			SWAP	Physio-hydrology	Rooting depth	Londo *et al.* (2001)
		Poplar	SECRETS	Physiology-based, photosynthesis	Allocation curve to partition green/woody	Deckmyn *et al.* (2004)
General	Cereals	Rice	ECOMERISTEM	Physiology-based, photosynthesis Sink–source interaction	Phenology (plastochron), RUE and respiration morphogenesis	Dingkuhn *et al.* (2005, 2006)

This is not intended to be a fully comprehensive list, but rather to reflect the different types of models available according to physiological, morphological and phenotypic complexity and their potential to accommodate genetic information.
RUE, radiation-use efficiency; LAI, leaf area index.

biomass. These examples serve to illustrate how energy yields can be improved by altering the yield or composition of the plant feedstock. For further examples see Chapple *et al.* (2007); Chen & Dixon (2007); Chang (2007).

Models provide powerful tools for investigating potential and actual growth under different conditions, and can be used to test hypotheses concerning the importance of altering different traits, represented in phenotypic and physiological parameters. A number of growth models have been developed for annual and perennial crops (Table 5), which vary in their degree of empiricism and number of parameters affecting yield. Empirical models are very useful in helping to predict yield at different sites (Lindroth & Bath, 1999; Clifton-Brown *et al.*, 2000; Suplick *et al.*, 2002). However, from the viewpoint of the target traits (Table 4), process-based models are more informative, as these embody hypotheses about growth mechanisms and are parameterized on the basis of independent experimental data (Ceulemans *et al.*, 1996; Isebrands *et al.*, 1996; Philippot, 1996; Deckmyn *et al.*, 2004). More research is needed in this area. In particular, there are no process-based models that integrate above- and belowground dynamics with respect to C and N for biomass crops. An even greater challenge will be to build a systems understanding of bioenergy production and link traits with corresponding gene pathways.

VII. Conclusions and perspectives

The examples reviewed in Section IV indicate the progress that has been made in identifying QTL and genes important for elevating bioenergy yields and optimizing DM composition, and in producing GM plants carrying altered bioenergy-relevant traits. However, there are still many challenges to overcome.

We leave others to debate whether GM bioenergy crops should be grown commercially. Here we acknowledge the vital role of GM in validating gene-trait associations and as potential tool for introducing qualitative differences, such as introducing resistance to pests and pathogens and overcoming some of the barriers to lignocellulosic recalcitrance (e.g. Chang, 2007). Nonetheless, many traits in Table 4 will not be easy to approach through a GM route, even through multiple gene introductions, and undesired associated effects may arise (Pedersen *et al.*, 2005). Moreover, while QTL mapping is becoming more accurate (Price, 2006), and genomic approaches, including alignment of maps to genome sequences, allow candidates to be identified, we are far removed from isolating the causal genes underlying most bioenergy yield traits.

It is essential that promises of what can be delivered though biotechnological approaches are tempered by realism and better understanding of how perennial crops are bred and grown. Too often, for researchers, the end point is a publication describing a GM plant or a QTL/gene, but this is not the end – it is only the beginning. The reality is that genetics has made only a partial contribution to the yield increases achieved to

date, more substantial gains having come from agronomy, or an interaction between agronomy and genetics (e.g. dwarfing genes and N in wheat). Crops will be grown not as individuals, but in the field, in cropping systems (Porter *et al.*, 2007), and the importance of assessing crop performance at different sites should not be underestimated. For perennial biomass crops, this is a considerable undertaking, and it will take several years for any new improvements to be realized. The advanced approaches that can be used today are possible only because of the continued commitment of individuals who have generated valuable information on performance through successive years of trials (e.g. Armstrong, 1997; Clifton-Brown *et al.*, 2001; Laureysens *et al.*, 2005; McLaughlin *et al.*, 2006). However, even when such trials are well conducted, they often overestimate yield, and it is essential to pay due attention to the difference between theoretical yield (obtainable by the plant under ideal and unlimited conditions); potential yield (obtainable in trial conditions); and actual yield (obtained in the field by farmers). During the green revolution, wheat grain yields in the UK doubled, part of a large international effort taking 30 yr (1950–80). However, yields have been static in the past decade, when molecular genetics and biotechnology could have influenced improvement. Substantial yield gains may well be obtained initially in perennial biomass crops (because of the relatively small effort devoted to breeding such crops in the past), but to propose even greater rates of yield improvement than have been achieved to date in annual arable crops is a bold claim that requires detailed substantiation.

The compelling argument in favour of growing perennial bioenergy crops revolves around the reduced competition with food, the higher energy savings, and greenhouse gas reductions, environmental sustainability and potential for higher DM production per unit of land. A considerable challenge has to be faced in elevating yields further as, unlike the yield advances in food crops, increased DM will have to be achieved without significantly increasing the requirement for inputs. The argument for utilizing marginal land also holds only if optimal yields can still be obtained when resources are limited. The efficient growth strategies of the perennial biomass grasses and trees rely on a pattern of partitioning of newly assimilated and recycled C and N between leaves, shoots and roots, resulting from a continually shifting balance between sources and sinks throughout the year. This balance is affected by biotic (pests and diseases) and abiotic stresses, especially water limitation. All the above need to be taken into consideration when attempting to increase/change C allocation in order to increase bioenergy from harvestable parts. We question whether GM approaches can take this into account fully.

In reviewing the traits associated with bioenergy crops in the context of sustainable production systems, we conclude that there are three main challenges facing yield improvement, which are interlinked:

- how to change thermal time sensitivity to extend the growing season
- how to increase aboveground biomass without depleting belowground biomass, so that sufficient reserves are still available for next year's growth (and thus without increasing the requirement for nutrient applications)
- how to increase aboveground biomass and not be limited by water.

Answers to these questions need to be found before projections of further yield enhancement are promised to policy-makers or the bioenergy industries.

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